[Reprint from the AMERICAN JOURNAL OF OPHTHALMOLOGY, March—April 15, 1885.]

# CELLOIDIN; THE NEW MATERIAL FOR EMBEDDING SPECIMENS FOR MICROSCOPIC SECTION CUTTING; ITS METHOD OF USE AND ITS ADVANTAGES.[1]

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The microscopic examination of the eye has always been hedged about by the extreme difficulty of the technique. Especially has this been the case when the examination of sections which cut through the entire eye or any considerable portion was necessary for the elucidation of the point to be examined. To cut through tissues varying in density from that of the sclera to that of the vitreous body has been the problem. To accomplish this some method of embedding is necessary, all of the methods heretofore in use employed the agency of heat to accomplish this, and thus became necessary water-baths, melting apparatus and a very considerable paraphernalia. In addition the tissue to be permeated all through equally should be kept in a moulten solution of the embedding fluid for from twenty-four to thirty-six hours before it is embedded; this, of course, necessitated the maintenance of heat just enough to keep the mass moulten for all this time; if the heat became too great the spec

1. My attention was first called to celloidin by a brief reference to a paper written on the subject by W. Jennings Milles in the British Medical Journal over a year ago, and after I had written to that gentleman on the subject he kindly replied in full as to his manner of using it. For many of the details of the method herein described I am indebted to Dr. C. S. Minot of the Harvard Histological Laboratory, where my work with this material has been done.

imen was destroyed, if not enough the process had to be recommenced.

All of these things cooperated to prevent microscopic examination of the eye being made, except at some large histological laboratory where all these conditions could be met. The expense of the apparatus and the large amount of room occupied by it effectually prevented the physician from attempting it either at home or in his office.

By the celloidin method of embedding all these objections are avoided. The only pieces of apparatus required are a few large bottles, corks and a few lead weights which any one can extemporize in a few hours. There is no temperature to be continually watched, there is no point in the whole process at which it cannot be immediately and safely discontinued and left with absolute security for weeks or months if necessary.

The whole process is done in closed bottles, so the danger of dust and overturning are reduced to a minimum; it produces no dirt and can be easily used in an office and put up on the closet shelves out of the way when not in actual process of manipulation.

But the great and crowning advantage of celloidin for use in embedding eyes lies in the fact that it is transparent, and so it is not necessary to dissolve out the embedding material in order to stain and mount the section.

The immense advantage of this can be readily seen, as the different tissues of the eye cut in some directions have no connection, so that when the embedding material is dissolved the section immediately falls apart and we can no longer distinguish the relationship of the various parts. The tissues can be stained without coloring the surrounding celloidin at all, and when the specimen is mounted the presence of the embedding material would never be surmised.

The celloidin used by me was manufactured by E. Schering, of Berlin, and comes in the form of cakes about four inches long, two and one half broad, and one-fourth of an inch thick; it is very hard, semi-transparent and resembles in appearance a piece of glue. It can also be bought cut up into fine shavings. To prepare this material

for use it is first necessary to cut or break a sufficient quantity into small pieces and then dissolve it in equal parts of ordinary strong alcohol (95 per cent) and sulphuric ether. It will take forty-eight hours to dissolve and then the solution should be about as thick as a heavy syrup. The proportion is about 14 grammes of celloidin to 100 cubic centimetres of the solvent.

A thin solution of the celloidin should be prepared by taking a portion of the above solution and by adding an equal part of the alcohol and ether mixture.

*Method of Embedding.*—Immediately upon the removal of the eye it should be placed in Mueller's fluid. The cornea and sclera should first be perforated to allow the access of the preserving fluid to all parts. When the eye has been sufficiently hardened a good method of removing the chromic acid is by simply putting the eye into a four per cent. aqueous solution of chloral hydrate and changing the fluid three or four times in several days. The advantage of this method for office work over that of washing under continuous stream will be obvious.

Next, if the vitreous is to be taken into consideration in the examination about to be made, it is best to freeze the eye and cut it through in an anterior-posterior direction. The specimen being prepared it is first placed in a mixture of equal parts of strong alcohol and ether to remove all traces of water. In this it is allowed to remain for twenty-four hours. In order to permeate the object thoroughly with the celloidin it is next placed into the second or thin solution above described. Here it is allowed to remain for thirty-six hours.

The box for embedding is most conveniently constructed in the following manner. Procure a *cylindrical* cork and wrap about it a piece of stout unruled writing paper in such a manner that it shall project beyond the end of the cork and so form the box. When the paper has been wound twice around the cork the end is secured by a pin thrust directly into the cork. To the lower end of the cork is then attached a leaden weight. The handiest method of accomplishing this is perhaps to insert the head of a pin into a bullet; the pin can then be thrust directly into the bottom of the cork and the weight is then firmly enough attached for all purposes.

The specimen is now placed into the box and the celloidin solution poured into it so as to fill it to two or three times the height of the specimen, and after waiting a few minutes for a film to form on the surface of the celloidin the entire box is submerged in alcohol of a specific gravity of .82.

When this is done often many bubbles of air are forced out of the cork and rise up through the celloidin; some of these are apt to become entangled in the specimen and become a source of annoyance subsequently. In order to avoid this it is a good plan to have the cork in the alcohol and weighted for some hours before the box is made. Another plan, although not so effective, is to cover the upper surface of the cork with a thin coating of celloidin and so to glaze it over before the box is made. At the end of twenty-four hours the paper should be removed and the cork with the embedding mass attached should be returned to the alcohol and allowed to remain until a suitable degree of density has been obtained for section cutting. If this process seems too slow it can be hastened by employing a lower per cent. alcohol, of say 60 to 75 per cent.

When sufficiently hardened the cork can be put in the holder of the microtome. In cutting the section a stream of alcohol should irrigate the knife. The sections are best removed from the knife by the aid of a camel's hair pencil. These sections will be entirely transparent even if the celloidin in the mass appears somewhat opaque.

The sections as fast as cut should be put into 90 per cent. alcohol. The surplus celloidin cut away from about the specimen can be returned to the strong solution and be used again.

A great advantage lies in the fact that the embedded specimen or the sections can be preserved without injury for months if kept in alcohol.

The sections can be mounted entire (embedding material and all.) The staining fluids do not color the celloidin. It does not do to attempt to clear up the specimen in the ordinary way with the oil of cloves as the celloidin contracts and spoils the specimen. The clearing up can be accomplished by following the ordinary methods and by using oil of bergamot instead of

the oil of cloves. The odor of the oil of bergamot would prevent this method in an office however.

A very good and rapid method of clearing up the specimen is as follows: Place the section after staining on the slide it is mounted on, and wash quickly but thoroughly with a few drops of *absolute* alcohol, drain it off and quickly add a few drops of chloroform, enough to entirely cover the section and add thereto drop by drop until the section is entirely clear; then just as the last of the chloroform evaporates from the section drop on the balsam. Another method which is exceedingly advantageous where a large number of sections of a specimen are to be mounted, is as follows: The stained section is placed on a slide and then a few drops of a thin solution of bleached shellac are dropped onto it. The slide is then put into an oven or other warm place free from dust and left until the shellac covering is perfectly hard; it is then treated with oil of cloves and covered.

If the specimen is of such a nature that it will not fall apart upon dissolving the celloidin the sections can be put into a mixture of alcohol and ether of equal parts for twenty-four hours and if sufficient solvent has been used the sections will be entirely freed from the celloidin. If it is for any reason desirable to free the section from the celloidin and at the same time it is from its nature liable to become disintegrated, the best method is to place the section upon a piece of rice paper and then add the ether and alcohol solvent after which the specimen can be easily transferred to the slide.